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SEP 78 W BOWERS, R HUBBARD, I LEAV, D WAGNER

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EFFECTS OF HYPERTHERMIA ON BILE PRODUCTION, ENZYME AND K^+ RELEASE, AND STRUCTURE OF PERFUSED RAT LIVER

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ABSTRACT

Isolated rat livers were perfused for 90 min. with oxygenated KRB at 37°C, oxygenated KRB at 43°C, or inadequately oxygenated KRB, containing low glucose, at 43°C. Bile production and GPT, GOT, and K^+ released into the perfusate were measured at zero time and every 15 min. All livers (8 per group) were processed for light and electron microscopy at the end of the 90 min. period. Control livers (37°C) were normal in all aspects monitored. Heat alone inhibited bile production after 45 min. and induced elevations in GPT, GOT and K^+ which reached peak levels in 60 min. Light and electron microscopic structure revealed focal hepatocellular damage and generalized endothelial damage. The addition of hypoxia and hypoglycemia to 43°C heat exposure inhibited bile production after 30 min. GPT, GOT, and K^+ release reached peak levels after 45 min. Structural changes were similar to those produced by heat alone except severe damage was uniformly distributed throughout the livers.

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Introduction

Focal hepatic necrosis is a common finding in both humans[1, 2, 3, 4, 5] and animals[6, 7] suffering from hyperthermia. These lesions have been described in cross country runners[3], Bantu miners[5], therapeutically hyperthermic patients[8] and in rats where hyperthermia was induced by either exhaustive exercise or sedentary heating[6, 7, 9].

Since the lesions were usually centrilobular, ischemic injury has been considered as a possible cause of the hepatic changes[7,10]. However, other causes, such as the direct effects of heated blood[2,11] and undefined toxins[12] on labile populations of cells, have been suggested. Unfortunately, studies concerning the pathogenesis of heat-induced hepatic necrosis have been hampered by the complexities of systemic manifestations occurring during heatstroke.

The isolated perfused liver, studied under precisely defined conditions, provides a means for evaluating the effects of individual or combined parameters on this organ. For example, Collins and Skibba[13] utilized this preparation to assess tolerance to the range of temperatures used in thermotherapy for cancer. In their work, metabolism of glucose, urea production and oxygen consumption declined between 42°C and 43°C. More recently, Collins et al.[14] observed a decrease in protein synthesis, RNA systhesis and DNA synthesis at 39°, 41° and 42°C, respectively.

This report, utilizing a similar method, compares clinical and pathological parameters of organs perfused at 43°C, and those made hypoxic and hypoglycemic while perfused at 43°C with control livers perfused at 37°C. Bile production, transaminase release, K^+ release, and light and electron microscopic structure were used as measures of hepatic integrity. The direct effects of heat and their amplification by hypoxia with hypoglycemia are discussed.

Materials and Methods

Livers were isolated from 24 Charles River CD strain male rats (mean weight 410g ± 61 SD) according to a procedure similar to that of Miller[15]; however, the bile duct was cannulated with a B-D 20 gauge 1-1/2 in. IV Cath connected to silastic tubing which allowed bile to drain into a tuberculin syringe with 0.01cc graduations. Just prior to ligation of the portal vein for cannulation, one cc of heparin (1,000 units/cc) was infused into this vein and allowed to circulate for one minute. The portal vein was then ligated and cannulated with a glass cannula which was connected to the perfusion apparatus at all times. The vena cava was then cut at the diaphram and perfusion was initiated allowing flow of oxygenated fluids throughout the extraction procedure. This reduced the time between portal ligation and actual perfusion. Another one cc of heparin was added to the perfusate entering the portal cannula.

The perfusion apparatus is illustrated in figure 1. Individual units were enclosed in water jackets for adequate temperature control. A 6 litre flask supplied warm oxygenated Krebs-Ringer bicarbonate, containing 0.14% glucose, to the reservoir (#1) on a continuous basis since the perfusate was not recirculated. The solution was oxygenated by bubbling 95% O_2 -5% CO_2 through spargers submerged in the fluid of both the reservoir and the 6 litre flask. The pH was adjusted to 7.4 with either 0.1N KOH or 0.1N HCL using an automatic titrator (Radiometer Inc.). Oxygenated fluid was pumped from the reservoir through a rice paper filter (#2) to a peristaltic pump. Fluid then flowed through a Y shaped manometer-bubble breaker (#3) which indicated perfusion pressure, allowed bubbles to escape, and the solution to bypass the liver when the perfusion pressure exceeded 26 cm of water. Perfusion pressure was recorded with Statham pressure transducer (#4), connected to a Grass model 7 recorder. Perfusing solution then circulated through a 10/30

glass joint (#5) designed to hold a Clark O_2 electrode, also connected to the recorder. The fluid proceeded through a small heating condenser (#6) connected to a glass portal cannula which was inserted into the portal vein of the liver. Perfusate exited the transected vena cava of the liver and out through the bottom of the perfusion chamber (#7) to another 10/30 glass joint (#8) containing a second Clark O_2 electrode. Finally, the fluid dripped into a collecting funnel leading to a collection vessel. The position of the gas humidifier (#9) is also shown in figure 1. A needle thermocouple, not showing, was inserted in the tubing attached to the portal cannula to monitor incoming temperature.

All connections between units were made with latex tubing. The temperature of the perfusing solution was controlled by adjusting five water baths which supplied the water jackets, and by adjusting current to a Clayborn heating tape (not shown) which was wrapped around the latex tubing leading from the condenser (#6) to the glass portal cannula. This arrangement allowed temperature control within $\pm$ 0.2°C. In order to insure that the changes observed in the liver were due to the experimental variables and not to changes induced in protein perfusing solution, albumin and red blood cells were not used. Schmucker et al.[16] and Goodman et al.[17] demonstrated that the perfused liver could be maintained without RBC with a flow rate about 30 ml/min. A flow rate of 40 ml/min. and a PO_2 above 500 mm Hg were employed with all but the hypoxic group.

A group of eight livers was perfused at 37°C and samples of perfusate exiting the livers, taken at 0 time and every 15 min. for 90 min., were analyzed for glutamic-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) using a Gilford 3402 automatic enzyme/end point analyzer and Worthington/Gilford Statzyme Kits. Potassium was measured with a Beckman Klina flame photometer. A reading of accumulated bile was taken every 15 minutes. At the end of the 90

minute period, the livers were either perfused with Karnowsky's fixative[18] with portions of the median lobe taken for light and electron microscopy, or separate slices were placed in neutral buffered formalin or 2% glutaraldehyde in cacodylate-sucrose buffer, and processed for light and electron microscopy. A second group of eight livers was perfused in the same manner as controls except the perfusing solution was maintained at 43°C. A third group of eight livers (hypoxic-hypoglycemic) was also perfused at 43°C, but the perfusing solution was oxygenated ($PO_2$57) with a gas containing 12% O_2, 5% CO_2 and 83% N_2. In this case, the perfusing solution contained only 0.0014% glucose and the flow rate was set at 10 ml/min. to further ensure the degree of hypoxia. Samples were taken as described for control livers.

Results

Figure 2 shows bile production of the three groups of perfused livers. Bars indicate SEM and points without them had SEM smaller than the point size. Control livers (37°C) produced bile in a linear manner over the course of the experiment. Bile production by the hyperthermic group (43°C) was reduced after 30 min. and reached a plateau after 45 min. The hypoxic-hypoglycemic hyperthermic group perfused at 43°C (HHH-43°) produced much less bile and reached a plateau after 30 min. Neither GPT nor GOT was detected in perfusate from control livers (Figs. 3 and 4). Perfusion at 43°C raised GPT and GOT levels after 45 min. with both reaching peak elevations at 60 min. The HHH-43°C group produced elevated GPT and GOT after only 30 min. and both enzymes reached peaks after 45 min. Since the flow rate of this group was reduced, the dotted lines show the curves corrected for the reduced flow rate.

As observed by others[17], control livers released potassium at a slow rate throughout the perfusion period (Fig. 5). The 43°C group released potassium at a

slightly higher rate which reached a peak at 60 min. As with the enzyme release, potassium release in the HHH-43°C livers was greater and reached a peak after 45 min.

With the exception of occasional dilation of sinusoids (Fig. 6), the light microscopic appearance of control livers was completely normal. Perfusion at 43°C for 90 min. resulted in dissociation of hepatocytes (Fig. 7). Necrosis of individual hepatocytes also occurred, but a zonal pattern was not discernible. The severity of these changes varied from one lobule to the next (Fig. 8). The HHH–43°C group (Fig. 9) showed changes similar to those produced by heat alone, but hepatocellular dissociation was more severe and generally involved the majority of lobules in a given section.

The ultrastructure of control (37°C) livers perfused for 90 min. was normal (Fig. 10). Both the heated (43°C) group (Fig. 11) and the HHH-43°C group (Fig. 12) were severely damaged. In both cases, endothelial cells were lost, microvilli were reduced in size and numbers, and cytoplasmic membranes of hepatocytes were frequently ruptured. Some hepatocytes in the group exposed to heat alone (43°C) retained nearly normal appearance with microvilli present, although sinusoidal endothelium was either diminished or missing. In both heated groups, mitochondria contained flocculent dense material.

Discussion

Hyperthermia in rats induces clinical[19], histological and ultrastructural changes[7] which are similar in many respects to those described in humans[2, 5]. This work demonstrates that heat alone (43°C) inhibits bile production after 45 min., and, at the same time, induces leakage of transaminases and K^+. According to these parameters, damage is maximal after sixty minutes at 43°C.

Throughout the experiments there were no indications of damage to control

livers except for slow leakage of potassium. Although Karnowsky's fixative was adequate for processing control tissue for both light and electron microscopy, it resulted in periportal shrinkage in the experimental livers. This was probably due to the effects of its high osmolarity on damaged cells. Fixation with glutaraldehyde for electron microscopy and formalin for light microscopy alleviated this problem, although this latter procedure necessitated use of tissue slices rather than perfusion fixation.

Tissues were processed for light and electron microscopy at the end of the experimental period (90 min.). At this time, dissociation of hepatocytes was a prominent feature at the light microscopic level, although some areas appeared to be nearly normal in the group exposed to heat alone. Electron microscopy revealed that endothelial cells were usually either damaged or missing even in areas where adjacent hepatocytes retained nearly normal structure. Thus, it appears that endothelial cells were injured early in the evolution of direct heat-induced hepatic injury.

The imposition of hypoxia and hypoglycemia, in addition to hyperthermia ($43^{\circ}C$), resulted in amplification of the same type of changes observed in livers exposed to heat alone. Bile production ceased 15 min. earlier than in those exposed to heat alone. This was followed by peak elevations of transaminases and K^{+}. In this group (HHH-$43^{\circ}C$), the histological and ultrastructural changes were essentially uniform throughout the lobes examined. These alterations differed from those induced by heat alone only in degree.

This observation that the addition of hypoxia and hypoglycemia changed only the extent of structural damage, and the time and extent of enzyme leakage indicates that either one or both of these additional stresses potentiate the effects of heat. Read et al.[20] indicated that hepatic anoxia augmented the toxic effects

of hepatic hyperthermia when dogs were subjected to extracorporeal liver perfusion. Hanisiadis et al.[21] also found that hypoxic cells were more sensitive to heat than aerobic cells, but Kim et al.[22] found no difference in the sensitivity of hypoxic and aerobic cells to heat. Since both the latter two conflicting reports were related to exposure of cultured cells to hyperthermia in cancer therapy, the evaluations of organ responses may be more relevant.

Kew et al.[5] and Willis et al.[8] described electron dense matrical material in mitochondria of hepatocytes from heatstroke victims and therapeutically hyperthermic patients which closely resembles that observed in mitochondria of this report. Flocculent mitochondrial densities are thought to represent denatured matrical protein and are commonly observed in cells which have been irreversibly injured[23].

The changes observed in heated isolated perfused livers are similar in many respects, although not identical, to those observed in both intact animals and in humans. Thus factors other than heat alone probably contribute to the character of the lesion in the intact animal, but different time/temperature relationships may also result in modification of the nature of the injury. This is supported by the work of Collins et al.[14] which indicated that different metabolic processes are altered at different temperatures.

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"In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council."

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Explanation of Figures

Fig. 1 - Perfusion apparatus: reservior 1, filter 2, bubble breaker-manometer 3, pressure transducer 4, receptor for O_2 electrode 5, warming condenser 6, perfusion chamber 7, receptor for O_2 electrode 8, gas humidifier 9.

Fig. 2 - Bile production by control livers was nearly linear over the perfusion period. Heat alone inhibited bile production after 45 min. The combined effects of heat, hypoxia and hypoglycemia (HHH) inhibited bile production after 30 min. Bars indicate SEM. No bars were used when the SEM was less than the point size.

Fig. 3 - Control livers released no detectable GPT. Heat alone induced release of GPT which reached a maximum after 60 min. Combined stressors heat, hypoxia and hypoglycemia (HHH), resulted in maximal release after 45 min. When flow was reduced with the HHH group, a proportional increase in detected enzyme occurred. The dotted line shows GPT curve for HHH group adjusted for flow rate.

Fig. 4 - Release of GOT follows a similar pattern to that of GPT.

Fig. 5 - Control livers released K^+ slowly over the 90 min. perfusion period. Peak levels for the two heated groups occurred at the same times as peak levels for both enzymes of respective groups.

Fig. 6 - Control liver, stained with H&E, has normal appearance (X330).

Fig. 7 - Heated liver, stained with H&E, shows a focal area of severe dissociation of hepatocytes (X270).

Fig. 8 - Heated liver, stained with H&E, showing area of milder damage (X230).

Fig. 9 - Heated hypoxic, hypoglycemic liver showing severe dissociation which occurs throughout the liver (X230).

Fig. 10 - Electron micrograph of control liver. Hepatocytes and endothelium are normal (X9,000).

Fig. 11 - Electron micrograph of heated liver showing severe damage. Electron dense material is present in what remains of mitochondria (X15,000).

Fig. 12 - Electron micrograph of heated, hypoxic hypoglycemic liver showing severe damage (X15,000).

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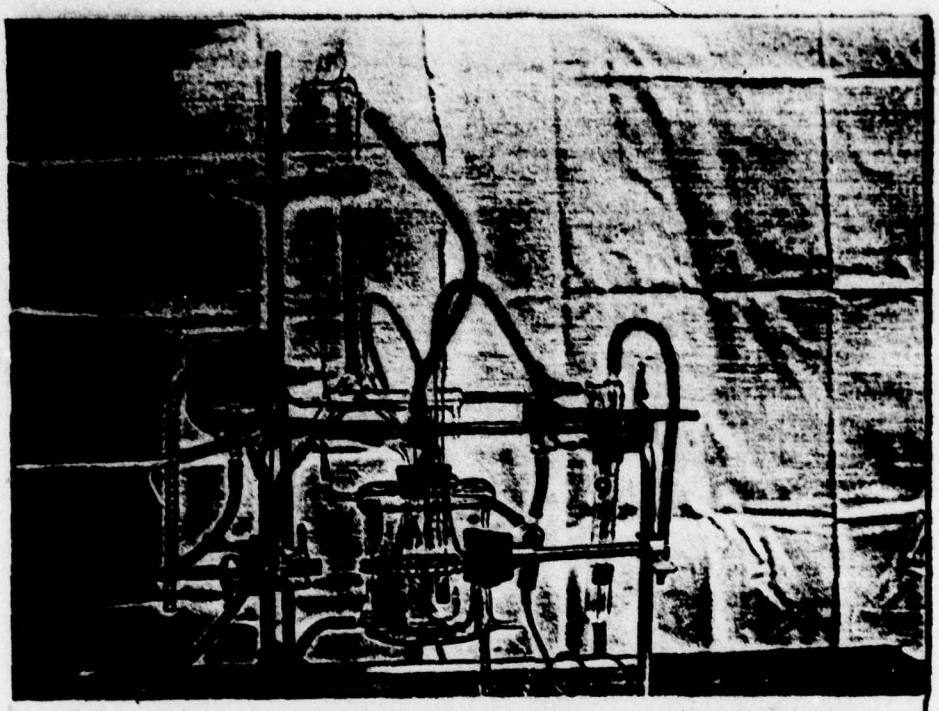

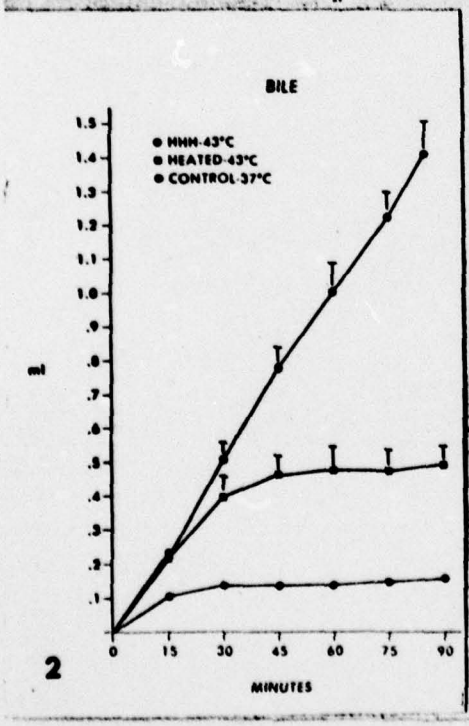
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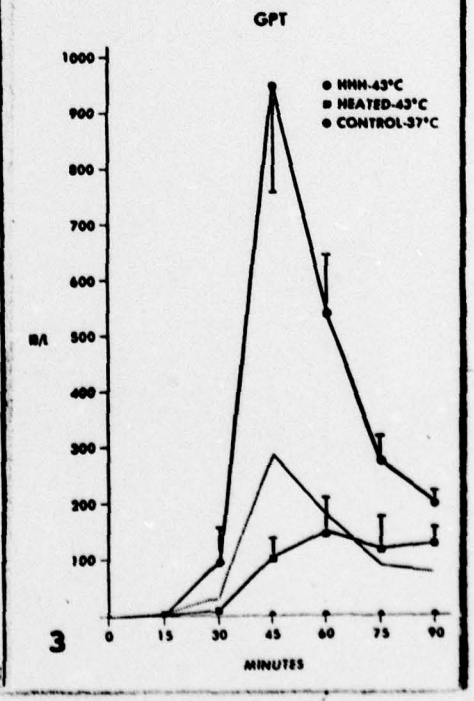
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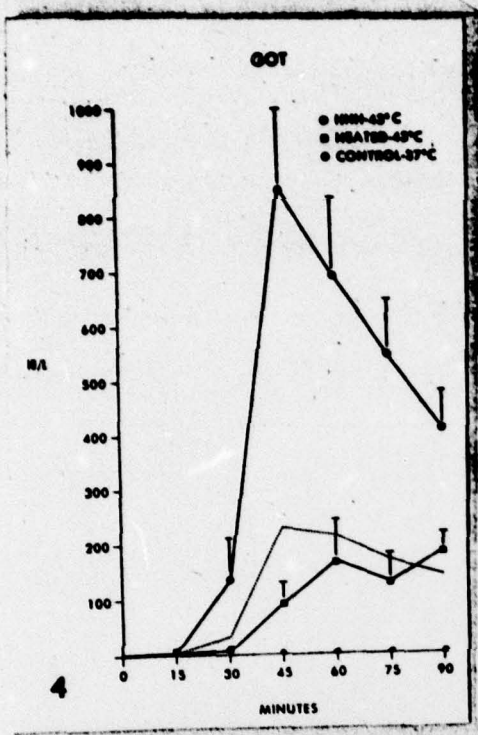
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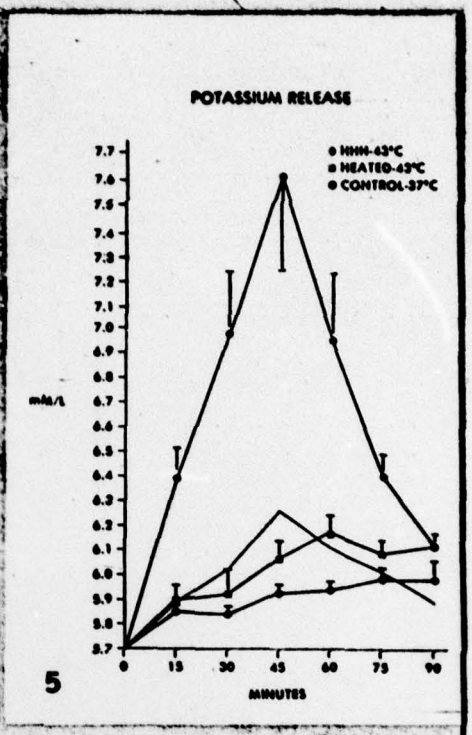
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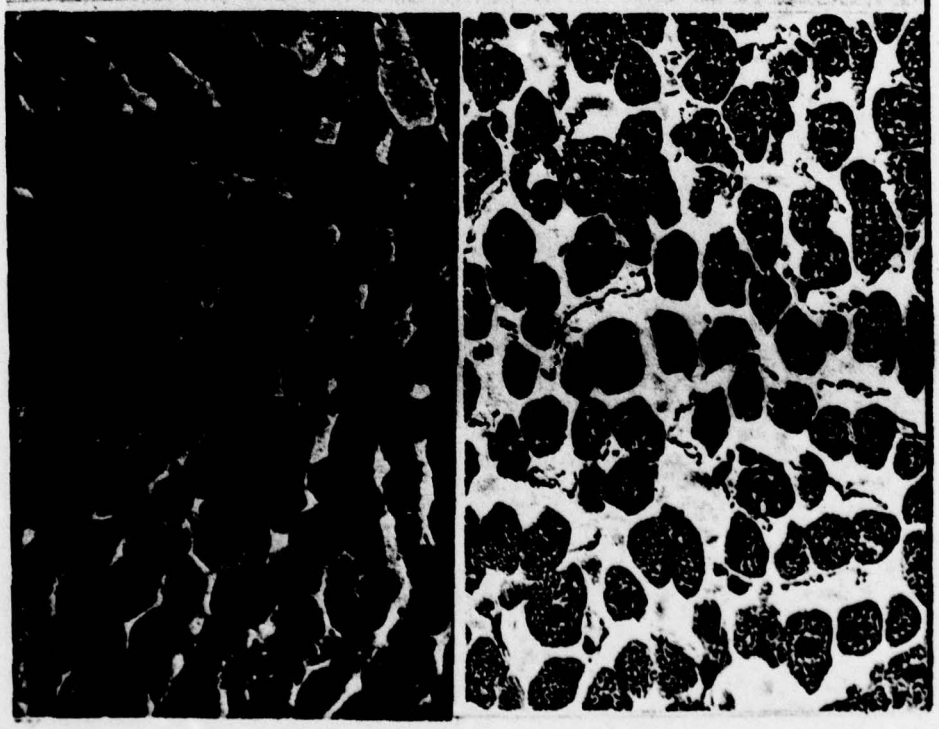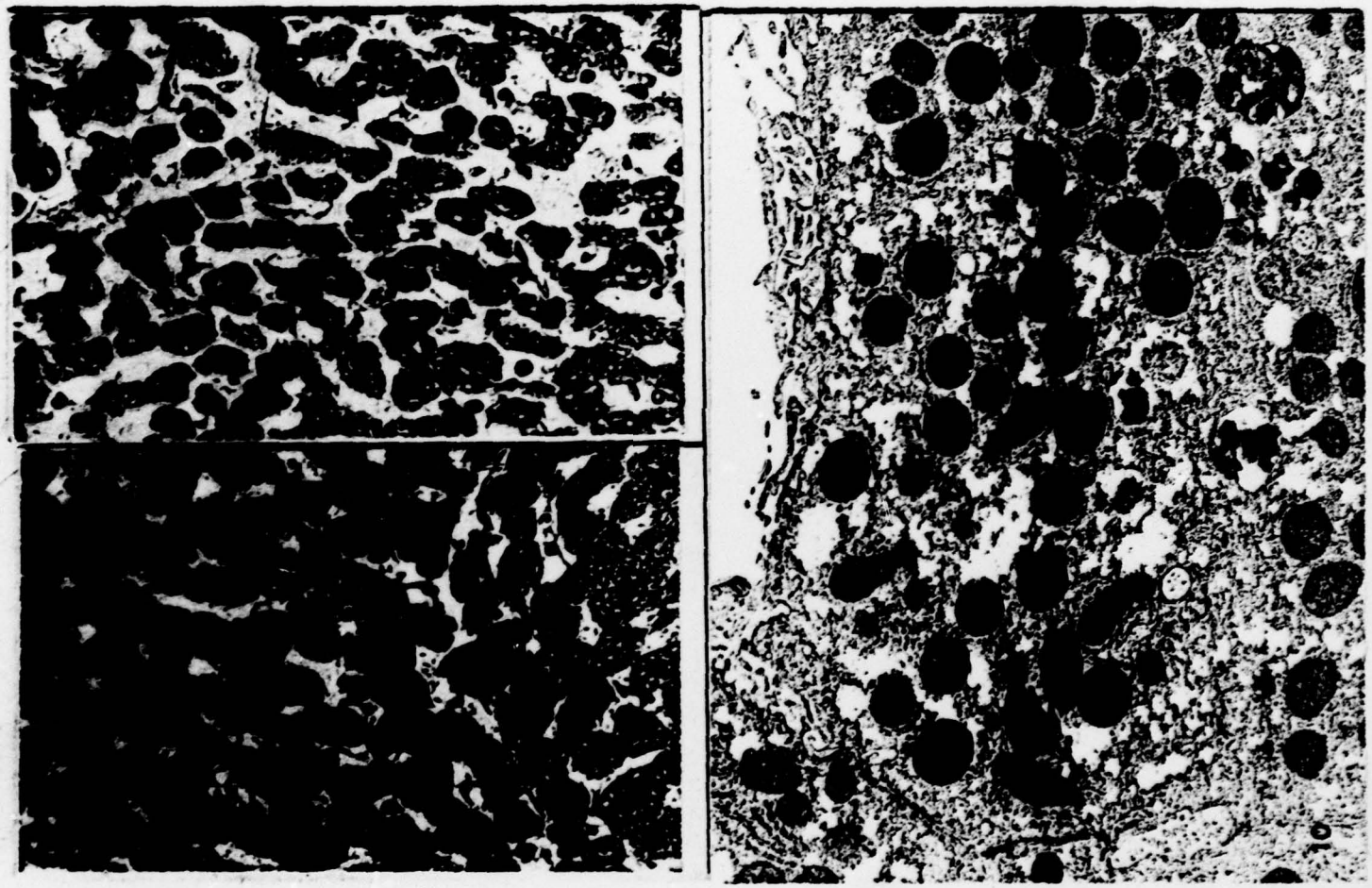
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